

were also compared with those obtained in CTX-M-15-producing *E. coli* clinical strains. Multilocus sequence typing (MLST) using the standard seven housekeeping loci was also performed. *E. coli* phylogenetic groups were identified by multiplex PCR. Presence of specific resistance genes was searched by PCR [blaOXA, blaTEM, blaCTX-M, aac(6')-Ib].

**Results:** Both CTX-M-15-producing *E. coli* belonged to phylogenetic group B2 and were clonally related by RAPD analysis. Moreover, they shared the same RAPD pattern of the predominant Portuguese *E. coli* clinical clone, producing CTX-M-15 and belonging to clonal complex ST131. Both strains were resistant to  $\beta$ -lactams (except carbapenems), tetracycline, kanamycin, tobramycin and ciprofloxacin. blaTEM-1 and aac(6')-Ib-cr-blaOXA-1 were also detected. Transfer of CTX-M-15 was achieved in both isolates.

**Conclusions:** Aquatic contamination with an epidemic and multiresistant B2 CTX-M-15-producing *E. coli* clone belonging to clonal complex ST131 is a matter of concern. This fact will undoubtedly increase the possibilities of dissemination of this epidemic clone and the corresponding blaCTX-M-15 plasmid. Control of release of these bacteria to the environment should be a public health priority in our country.

**P1526 Environmental emergence of multiresistant Enterobacteriaceae harbouring blaCTX-M-15, and aac(6')-Ib-cr in Portugal**

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**Objectives:** Since our previous reports of seawater contamination with ESBL producers, we noticed a change in resistance phenotype in the last years, with emergence of CTX-M profile, in 2004, and increased resistance to fluorquinolones, in 2006. Spreading of CTX-M-15 producers in the clinical setting, has already been reported in Portugal in association with TEM-1, OXA-1 and AAC-(6')-Ib-cr. In that way, it was our purpose the detection of CTX-M-15 and AAC-(6')-Ib-cr producing isolates, in pluvial water streams reaching the shore and coastal seawater.

**Methods:** Pluvial water streams reaching the sea and seawater were collected from 2004 to 2006, from 3 beaches of the Porto area, in Portugal. Isolates were selected by membrane filtration technique and the filters were placed on MacConkey agar with and without ceftazidime (2 mg/l) or cefotaxime (2 mg/l). Lactose fermenters were randomly selected and susceptibility was determined according to the CLSI guidelines. Screening for ESBL producers was performed by the double disk synergy test. Identification was achieved by ID 32 GN.  $\beta$ -lactamases were characterised by isoelectric focusing and, in representative isolates, were identified by PCR and sequencing. Conjugation assays were performed with *Escherichia coli* HB101 and K802N.

**Results:** Forty six multiresistant Enterobacteriaceae isolates (mostly *E. coli* and *Klebsiella pneumoniae*), producing CTX-M-15 in different combinations with TEM-1, OXA-1 and AAC-(6')-Ib-cr, were recovered from pluvial water streams presenting faecal contamination and seawater. Fifteen isolates were able to transfer the CTX-M-15 gene, by conjugation.

**Conclusion:** The presence of CTX-M-15 and AAC-(6')-Ib-cr producers, in pluvial water streams and seawater, seems to reflect unexpected contamination by wastewater related to the healthcare setting, like hospitals, tertiary care institutions, long term care and nursing homes. This situation seems relevant in terms of public health and environmental protection. The incoming of this kind of multiresistant ESBL producers to natural environments and the transferability of the ESBL gene by conjugation, might provide a track for environmental dissemination of resistant bacteria and genes that may create a source of transferable traits for environmental bacteria, influencing natural reservoirs of resistance with possible transference for emerging pathogens.

**P1527 Molecular epidemiology of plasmid-mediated quinolone resistance determinants in extended-spectrum  $\beta$ -lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from Turkey**

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**Objectives:** To evaluate the distribution of plasmid-mediated quinolone resistance (PMQR) of the Qnr and aminoglycoside acetyltransferase AAC(6')-Ib-cr types, among clinically-extended-spectrum  $\beta$ -lactamase (ESBL) producing enterobacterial isolates recovered from blood cultures of patients in different Turkish hospitals.

**Methods:** 248 ESBL-producing *Escherichia coli* and *Klebsiella* sp. isolates were collected from blood cultures from January to December 2006 in nine Turkish hospitals. ESBL-positive isolates were detected according to Clinical and Laboratory Standards Institute (CLSI) criteria. The bacterial isolates were tested for qnrA, qnrB, qnrS and aac(6')-Ib-cr genes by a PCR technique.  $\beta$ -Lactamase-encoding genes were detected by PCR using primers for detection of TEM, SHV, OXA and CTX-M variants. Conjugation experiments were performed to determine whether the plasmids carrying the qnr or aac(6')-Ib-cr genes were self-transferable. Genetic structures surrounding the qnr gene were analysed by PCR and cloning.

**Results:** A total of 138 and 110 ESBL-producing isolates were identified as being *E. coli* and *K. pneumoniae* sp., respectively. Sixty-three percent of the 248 ESBL-producing isolates were resistant to nalidixic acid. Multiplex PCR-screening detected a single *K. pneumoniae* isolate harbouring a qnrB1 gene (0.4%), whereas no qnrA or qnrS gene was detected. The qnrB1-positive isolate was also positive for the blaCTX-M-15, blaSHV-12 and aac(6')-Ib-cr genes. The qnrB1-positive plasmid was 124-kb in size, co-harboured the blaSHV-12 gene, and attempts to transfer by conjugation failed. The blaCTX-M-15-positive plasmid co-harboured the aac(6')-Ib-cr gene. Out of 50 ESBL-producing isolates tested, 39 (78%) were positive for the aac(6')-Ib-cr variant. All isolates carrying the aac(6')-Ib-cr gene were resistant to ciprofloxacin and two of them, two *K. pneumoniae* isolates, were resistant to imipenem due to the production of  $\beta$ -lactamase OXA-48.

**Conclusion:** This study constitutes an epidemiological survey of PMQR determinants among ESBL enterobacterial isolates from highly significant clinical samples (blood isolates) and shows a high prevalence of AAC(6')-Ib-cr determinants in Turkey. However Qnr determinants are very rarely identified here.

**P1528 Low prevalence of plasmid-mediated quinolone resistance in Norwegian and Swedish clinical isolates of *Escherichia coli* and *Klebsiella* spp.**

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**Objectives:** The objective of this study was to examine the prevalence of the plasmid-mediated resistance genes, qnr and aac(6')-Ib-cr, among Norwegian and Swedish clinical isolates of *Escherichia coli* and *Klebsiella* spp. with reduced susceptibility to ciprofloxacin and/or resistance to nalidixic acid.

**Methods:** 487 isolates of *E. coli* (n=326) and *Klebsiella* spp. (n=31) from (i) Kronoberg Sweden (n=352) isolated between 2004–5, (ii) the Norwegian surveillance programme for antimicrobial resistance (NORM) (n=318) isolated in 2005 and (iii) ESBL- (n=304) or AmpC-producing (n=33) isolates from the Norwegian Reference Centre for Detection of Antimicrobial Resistance (K-res) collected between 2003–5, were screened for the presence of qnr and aac(6')-Ib-cr genes by multiplex and single PCRs, respectively. PCR products were confirmed by sequencing and the aac(6')-Ib-cr variant was also identified by BstCI-digestion. The genetic environment of qnrS positive isolates was examined by targeted PCR and sequence analysis. Transfer of qnr to *E. coli* J53 RifR was examined.